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SSR markers closely linked to the *Pi-z* locus are useful for selection of blast resistance in a broad array of rice germplasm

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Abstract

Pi-z is a disease resistance gene that has been effectively used to combat a broad-spectrum of races of the rice blast fungus *Magnaporthe grisea*. Although DNA markers have been reported for selection of the *Pi2(t)* and *Pi-z* resistance genes at the *Pi-z* locus, markers that are more tightly linked to the *Pi-z* locus would benefit rapid and effective cultivar development. Analysis of the publicly available genome sequence of Nipponbare near the *Pi-z* locus revealed numerous SSRs that could be converted into markers. Three SSRs on rice PAC AP005659 were found to be very tightly linked to the *Pi-z* locus, with one marker, AP5659-3, co-segregating with the *Pi-z* resistance reaction. The *Pi-z* factor conferring resistance to two races of blast was mapped to a 57 kb region on the physical map of Nipponbare in a location where the *Pi2(t)* gene was physically mapped. Two SSR marker haplotypes were unique for cultivars carrying the *Pi-z* gene, which indicates these markers are useful for selection of resistance genes at the *Pi-z* locus in rice germplasm.

Abbreviations: SSR – simple sequence repeat; BAC/PAC – bacterial/P1-derived artificial chromosome

Introduction

The rice blast disease, caused by *Magnaporthe grisea* Cav. [asexual form known as *Pyricularia grisea* (Cooke) Sacc.], is one of the most serious fungal diseases threatening world rice production. Genetic resistance to rice blast has been and continues to be extensively used by rice breeders and pathologists to combat this disease. Numerous races of the fungus exist and blast resistance genes, commonly called *Pi* genes, providing a broad spectrum of resistance against the most prevalent races can be extremely valuable in rice breeding efforts.

The *Pi-z* gene, first identified by Kiyosawa (1967) in the USA medium grain cultivar Zenith, is a broad spectrum *Pi* gene that has been effectively used by rice researchers throughout the world. The *Pi-z* gene has been mapped on rice chromosome 6 and found to be allelic to the *Pi2(t)* resistance gene (Inukai et al. 1994) that maps near the centromere of this chromosome (Yu et al. 1991). The *Pi-z*^{t} and *Pi9(t)* genes also map to the same area on rice chromosome 6 (Liu et al. 2002; Hayashi et al. 2004; Zhou et al. 2004) and appear to be alleles at the *Pi-z* locus.

DNA markers have been identified to help incorporate blast resistance factors at the *Pi-z* locus into improved cultivars using marker assisted selection. Markers suitable for selection at this locus included ones based on restriction enzyme

digestion of PCR amplification products being linked to the *Pi2(t)* gene (Hittalmani et al. 1995), dominant PCR markers based on resistant gene analog sequences and codominant simple sequence repeat (SSR, also know as microsatellite) markers linked to the *Pi-z* gene (Conaway-Bormans et al. 2003), and single nucleotide polymorphism (SNP) markers linked to the *Pi-z* and *Pi-z*^{t} genes (Hayashi et al. 2004). However, the SSR markers developed by Conaway-Bormans et al. (2003) were not tightly linked (> 2.5 cM) to the *Pi-z* gene and only flanked *Pi-z* on one side. Since SSR markers are widely used and continue to be evaluated in DNA marker laboratories, we investigated the possibility of developing more tightly liked SSR markers for the *Pi-z* locus using the release of more sequence information on rice chromosome 6 by the Rice Genome Research Program (RGP) in Japan. As a result of analyzing public genome sequence information, designing SSR markers, and testing marker association with disease reactions, we present newly developed SSR markers tightly linked to and flanking both sides of the *Pi-z* locus that are well suited for molecular breeding.

Materials and methods

Mapping populations

Three genetic populations segregating for the *Pi-z* resistance gene were studied for developing markers at or near the *Pi-z* locus. The cultivars carrying the *Pi-z* gene, which confers resistance to races IC-17 and IE-1k, in these studies were Bengal (USDA-ARS-GRIN PI 561735) or Panda (PI 560299) which were crossed to the blast susceptible cultivars Maybelle, Cypress, or M-205, all of which lack the *Pi-z* gene. One population was composed of 371 F_2:F_3 families from a Maybelle*2/Bengal cross segregating for resistance to blast race IC-17 that was genotyped at the F_2 level and progeny tested by phenotyping the F_3 generation. A Panda/M205 population composed of 405 F_3 progeny (derived from 143 F_2 families) was genotyped and phenotyped for resistance to blast race IC-17. In addition, a population of 201 F_5 families derived by single panicle descent from a Cypress/Panda cross was genotyped at the F_8 level and phenotyped at the F_9 generation for resistance to blast race IE-1k.

Disease resistance screening

Cultures of blast isolates 93M25 of race IE-1k and 75T38 of race IC-17 (Marchetti et al. 1987) maintained at the USDA-ARS Rice Research Unit (RRU) at Beaumont, Texas were used to inoculate parents, progeny, and controls of resistant and susceptible cultivars to test for their disease reaction. Standard seedling inoculation methods were used to evaluate plants for their disease resistance reaction using a scale from 0 to 9 (Marchetti et al. 1987). Plants scored with a reaction of less than 3 were rated as resistant whereas plants with a reaction greater than 3 were rated as susceptible. Plants with a reaction of 3 were considered inconclusive and were left out of subsequent genetic analyses, although it can be noted that such plants were infrequent. Leaves were harvested from phenotyped plants, frozen in a −80 °C freezer, lyophilized, and then stored at −20 °C prior to DNA isolation. For germplasm screens, blast isolates 75L5 (USDA-ARS RRU identification number) of race IB-45, 793 of race IB-49, 429 of race IB-54, 75T38 of race IC-17, 93M25 of race IE-1k, 74T3 of race IG-1, and 74L2 of race IH-1 (Marchetti et al. 1987) were used to determine the presence of the *Pi-z* gene in germplasm obtained from breeder and foundation seed sources, the USDA-ARS-National Small Grains Collection (Aberdeen, ID), or the International Rice Research Institute-Genetic Resources Center (Los Baños, Philippines).

Microsatellite marker development

Publicly available DNA sequence information from BAC/PAC sequences at or near the *Pi-z* locus was downloaded from GenBank (http://www.ncbi. nlm.nih.gov/Genbank/index.html/) and scanned for SSR regions, primarily by word-processor search commands. SSR sequence searches were concentrated primarily on AC, AG, CG, CT, AAC, ACC, AAG, AGG, AAT, ATT, CCT, CTT, GGT, GTT, ACG, ACT, AGC, AGT, ATC, ATG, CGT, CTG, ATAC, ATAG, ATCT, and ATGT repeats. PCR primers for amplifying SSR markers were designed by using online software found at the Primer3 website (Rozen and Skaletsky 2000; http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and subjectively choosing

(based on degree of primer self-complementarity, lack of nucleotide repeats in primer sequences, and presence of all four nucleotides in the primer region) primers flanking the repeat region. Primers were named after the GenBank sequence accessions they were identified from (e.g., marker AP4791 was named after GenBank accession AP004791). The parameter settings for primer design included using 56 °C (± 2 °C) as the primer annealing temperature (T_m), max poly-X = 4 bases, C/G clamp of one base, and using the default settings for the remaining parameters. Primers were obtained from various commercial vendors and tested for amplification quality and polymorphism among parental lines used in *Pi-z* mapping studies. Robust primers providing unambiguous, strongly expressed, and polymorphic amplification products were fluorescently labeled for further marker analysis. Markers RM527 and RM6836, previously found to be near the *Pi-z* locus (Conaway-Bormans et al. 2003), were obtained from the Gramene project website (http://www.gramene.org/; Ware et al. 2002) and also included in primer testing.

Marker analysis

DNA was isolated from 20 to 40 mg lyophilized leaf tissue cut up into small (~4 mm^2) pieces and put into 2 ml microcentrifuge tubes. A 900 μl aliquot of PEX/CTAB extraction buffer containing 6.25 mmol potassium ethyl xanthogenate, 0.5% cetyltrimethyl ammonium bromide (CTAB), 700 mmol NaCl, 10 mmol EDTA, and 100 mmol Tris (pH 7.5) was added to each sample tube and lightly vortexed. Tubes were placed in a 65 °C water bath for 1–1.5 h, cooled briefly, and extracted with 700 μl 100% chloroform. Tubes were centrifuged at top speed in a microfuge for 10 min, the aqueous layer retained, and 800 μl of isopropanol was added to precipitate nucleic acids. Nucleic acid pellets were washed with 400 μl 100% ethanol, dried, and resuspended in 100 μl TE buffer [10 mmol Tris (pH 7.5), 0.5 mmol EDTA].

SSR markers were PCR-amplified in 10 μl volumes containing 55 mmol Tris (pH 9.0), 45 mmol $(NH_4)_2SO_4$, 2.0 mmol $MgCl_2$, 0.2 mmol dNTPs, 0.1 μmol each of forward and reverse primers (Table 1), 1.0 μl of DNA extract containing approximately 2.5 ng DNA (not quantified for most samples), and 0.3 U *Tfl* polymerase (Epicentre Technologies, Madison). Amplification reaction conditions used an initial denaturation step at 94 °C for 2 min, followed by 25 cycles of amplification at 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 1 min, and a final extension at 72 °C for 2 min.

Non-fluorescently labeled amplification products were assayed for parental polymorphism in non-denaturing 8% polyacrylamide (29:1, acryl:bis) 1× Tris–borate gels (TBE), run overnight at 220 V. Gels were stained directly on separated, water-rinsed plates for 10 min with 50 ml solution of GelStar nucleic acid stain (Bio Whittaker Medical Applications, Rockland, MA) diluted 10,000 fold in 1× TBE. Stained plates were rinsed with water and placed on DarkReader transilluminator (Clare Chemical Research, Denver, CO), DNA banding patterns were photographed with a digital camera, and images were downloaded onto a desktop computer.

Table 1. SSR primer sequences and physical locations in the sequenced rice genome.

Marker	Forward	Reverse	Start[a]	Stop	Length[b]
RM6836	TGTTGCATATGGTGCTATTTGA	GATACGGCTTCTAGGCCAAA	9308927	9309104	178
AP3540	TTTGCCATCAATTTTCATTC	TCAACTCTCCTCCCTACAGC	9575635	9575877	243
RM527	GGCTCGATCTAGAAAATCCG	GGCTCGATCTAGAAAATCCG	9862290	9862522	233
AP4791	AAACGGAGGGAGTACATTG	GGATCGTCGATTTGATTTG	10093246	10093556	311
AP5930	CATGAAAGAAAGGAGTGCAG	ACAGAATTGACCAGCCAAG	10278000	10278154	155
AP5659-5	CTCCTTCAGCTGCTCCTC	TGATGACTTCCAAACGGTAG	10357166	10357453	288
AP5659-3	TCTTTCCTAGGGAACCAAAG	AAGTAGTTGCTGAGCCATTG	10406597	10406825	229
AP5659-1	TGCTGAGATAGCCGAGAAATC	ACTAGCTGCCCACCTAAGC	10414829	10415031	202
AP5413	GAAAGTGGGTTAAGGGACAC	GAGTCTGTCAAGATTAAGATTCAG	10993171	10993504	334
AP4007	CGACGAACAACAACTCAAC	GTTCTCTCGGTTTGGACTTC	11243159	11243338	180

[a]Physical locations determined from Release 3, TIGR Rice Pseudomolecules, http://www.tigr.org/tdb/e2k1/osa1/pseudomolecules/info.shtml.

[b]Nucleotide (nt) chain length of Nipponbare sequence determined from GenBank database information.

Fluorescently-labeled amplification products were analyzed by capillary electrophoresis, where 1 μl of PCR product was diluted into 99 μl water. One microlitre of this mixture was diluted into 9 μl of a formamide-400HD ROX mixture (ABI, Foster City, CA), placed in a 96 well microplate, denatured for 2 min at 94 °C, immediately cooled to 4 °C, and placed in the a capillary electrophoresis instrument (3100 Genetic Analyzer, ABI). Samples were run with POP-4 matrix using the GeneScan36 POP4 Default Module, with run conditions of 60 °C, 15 kV, and 25 min. Fragment size data were automatically stored on a computer and analyzed using Genotyper Version 3.7 software (ABI) to determine progeny genotypes.

Genetic linkages among SSR marker genotypes and disease resistance phenotypes or genotypes were determined using Map Manager QTX (Manly et al. 2001). Disease resistance phenotype was scored as a dominant trait (where plants homozygous for disease resistance are not differentiated from heterozygous plants) in the Panda/M-205 and Cypress/Panda populations. However, the Maybelle*2/Bengal F_2 population was genotyped for disease resistance by F_3 family analysis, where homozygous and heterozygous plants are differentiated. Recombination estimates for different generations of progeny (i.e., F_2, F_3, and F_8) were taken into account using the advanced intercross option from the linkage evaluation menu of Map Manager QTX. Recombination distance data (Morgan map units) between resistance genes and markers from separate crosses were combined by averaging distances weighted according to the number of progeny.

Results

Candidate SSR marker identification

DNA sequences from 20 BAC and PAC accessions surrounding the *Pi-z* locus were obtained from public database sources, primarily due to the sequencing efforts of the Japanese Rice Genome Sequencing Project (http://rgp.dna.affrc.go.jp/), to find suitable SSR markers for mapping. Word-processor text searches for 10 or more SSR repeats were performed within this sequence information to identify candidate SSR markers. SSRs with AT repeats were rarely used as candidates, since PCR amplification through AT rich regions is frequently unsuccessful (Temnykh et al. 2001). As the *Pi-z* gene location became better resolved through mapping, searches for as few as seven SSR repeats were used to identify candidate markers tightly linked to the *Pi-z* gene. Seventy primer pair combinations were designed and over 72 primer combinations were tested for amplification quality and polymorphism among the *Pi-z* parents. Nineteen primer pairs failed to give amplification products, including two candidate primer pairs flanking SSRs with AT repeats. Eighteen primer pairs produced non-polymorphic amplification products among the parents involved in the crosses studied, and 34 primer pairs produced at least one polymorphism between these parents. Fourteen SSR primer pairs, including two obtained from the Gramene project website (http://www.gramene.org/), were suitably functional, giving good amplification signal and consistent polymorphism between the parents in two or all three of the analyzed crosses, and were fluorescently labeled for subsequent mapping.

Mapping of SSR markers

Previously published disease reaction data showed that the *Pi-z* gene confers resistance to the common USA blast races IC-17, IE-1 (including IE-1k), IG-1, and IH-1 (Marchetti et al. 1987; McClung 2002). All IB blast races are virulent against *Pi-z*, including the common USA races IB-45, IB-49, and IB-54, as well as the less-common USA races IB-1 and IB-33. Previous knowledge of race resistance reactions obtained from USA Uniform Regional Rice Nursery and USDA disease reaction data indicated that Bengal and Panda possess the *Pi-z* gene and that Maybelle, Cypress, and M-205 lack *Pi-z* (Conaway-Bormans et al. 2003; Marchetti, unpublished results). Maybelle and M-205 are susceptible to all USA blast races and appear to carry no blast resistance genes. However, Cypress appears to possess the *Pi-k^h* resistance gene, which confers resistance to blast races IB-45, IB-54, IG-1, and IH-1, as well as the *Pi-i* resistance gene, which confers resistance to blast race IH-1 only (Marchetti et al. 1987; McClung 2002). Therefore, the use of these three genetic populations permitted mapping of the *Pi-z*

gene relative to the SSR markers when screened with races IC-17 and IE-1k.

All 10 SSR markers presented in Table 1 were polymorphic in the Maybelle*2/Bengal cross, and nine SSR markers were used to map the *Pi-z* genetic factor conferring resistance to blast race IC-17. The IC-17 resistance factor mapped very closely to the marker AP5659-3, with no recombinants between this marker and the disease reaction (see Table 2 for all marker results presented below). There was only one recombinant progeny between IC-17 resistance and marker AP5659-1 and one recombinant between IC-17 resistance and AP5659-5. Also, any recombination between all tested markers 'upstream' or 'downstream' from marker AP5659-3 was associated with a concurrent recombination with disease resistance. Progeny testing among 16 families showing recombination between markers RM6836 and AP4007 indicated that IE-1k resistance also co-segregated with AP5659-3 in this population (data not shown).

Table 2. Segregation of the *Pi-z* gene disease reaction with SSR markers found near the *Pi-z* locus.

Cross	SSR marker	Recombinants	Distance (cM)
Maybelle*2/Bengal	RM6836	11/300	1.8
	RM527	6/371	0.8
	AP4791	4/371	0.5
	AP5930	2/371	0.3
	AP5659-5	1/371	0.1
	AP5659-3	0/371	0.0
	AP5659-1	1/371	0.1
	AP5413	4/371	0.5
	AP4007	12/367	1.6
Panda/M-205	RM6836	12/405	1.5
	AP3540	10/405	1.2
	RM527	6/405	0.7
	AP4791	5/405	0.6
	AP5930	0/405	0.0
	AP5659-5	0/405	0.0
	AP5659-1	1/405	0.1
	AP5413	9/388	1.2
Cypress/Panda	AP3540	4/200	1.0
	AP4791	1/199	0.3
	AP5930	0/194	0.0
	AP5659-5	0/192	0.0
	AP5659-3	0/192	0.0
	AP5659-1	0/192	0.0
	AP5413	6/198	1.5

Eight of the SSR markers were polymorphic in the Panda/M-205 cross, while AP5659-3 and AP4007 were not segregating in this population. IC-17 resistance co-segregated with markers AP5659-5 and AP5930 and only one recombinant was found between AP5659-1 and IC-17 resistance. Any recombination observed between a marker and AP5659-5 or AP5930 was associated with a recombination between that marker and the disease reaction.

Seven SSR markers were also polymorphic in the Cypress/Panda cross, whereas markers RM5836, RM527, and AP4007 were monomorphic in this population. IE-1k resistance co-segregated with SSR markers AP5659-1, AP5659-3, AP5659-5, and AP5930, so it was difficult to map the IE-1k resistance factor within this marker region for this cross. Any recombinants outside this region showed a concurrent recombination with the IE-1k disease reaction.

The combined segregation information of 977 progeny lines from all three cross populations was used to develop a map of the *Pi-z* locus (Figure 1). On this map, where the resistance reaction co-segregates with AP5659-3 and is closely linked and flanked by AP5659-1 and AP5659-5, it can be seen that the *Pi-z* resistance factor conferring IC-17 or IE-1k resistance can be localized to a 57 kb region on the Nipponbare physical map. Annotated sequence data shows that there are several disease resistance gene analogs (RGAs) on the BAC from which these three markers were identified (GenBank accession AP005659; see http://www.tigr.org/tigr-scripts/e2k1/irgsp_nbac_display.spl?db=osa1&asmbl_id=9871). In this annotation, there appears to be one RGA between markers AP5659-1 and AP5659-3 and two RGAs between markers AP5659-3 and AP5659-5. These three SSR markers are all in the 337 kb region flanked by the SNP markers z4792 and z60510 identified by Hayashi et al. (2004) that are most closely linked to the *Pi-z* gene (Figure 1). The comparison of genetic (recombination) distance to physical distance in the crosses we analyzed indicates that 1.0 cM is equivalent to 593 kb (Figure 1).

Pi-z *marker polymorphism in rice germplasm*

The *Pi-z* markers we developed were analyzed in 130 widely-used USA and international rice

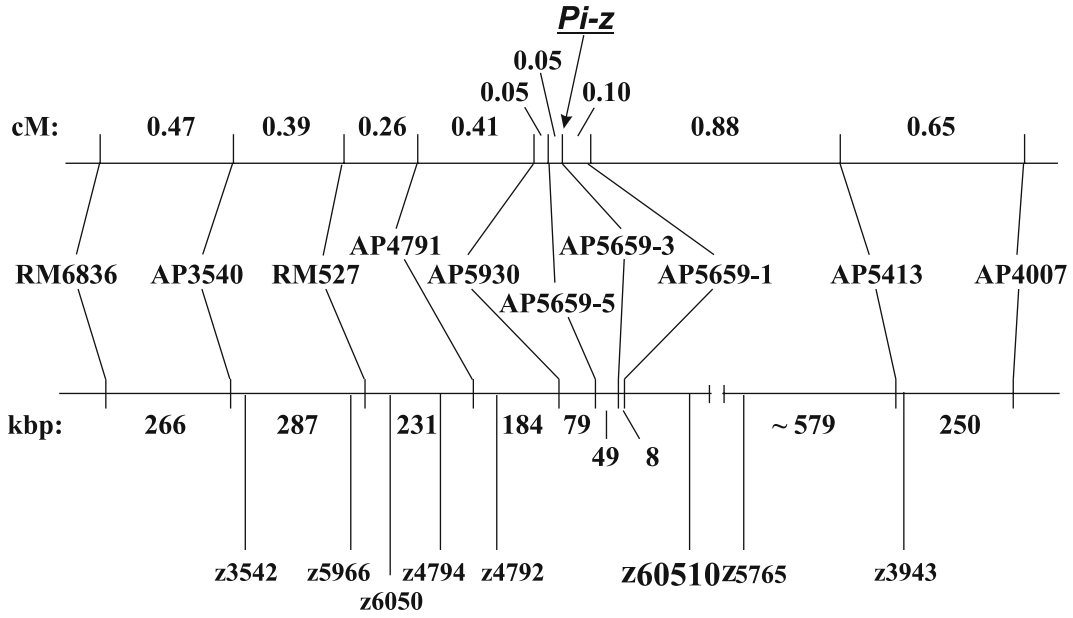

Figure 1. Genetic and physical map of the *Pi-z* gene and markers. The genetic map, displayed on top, is presented with centimorgan map distance shown between the SSR markers. The physical map of the *Pi-z* SSR markers, displayed on bottom, shows the genetic distance in kb between the *Pi-z* markers in the Nipponbare genomic sequence. The SNP markers of Hayashi et al. (2004) are shown at the very bottom, with lines displaying their respective physical map positions.

accessions to survey SSR marker associations with the *Pi-z* gene. A subset of 40 germplasm representative of the SSR genotypes observed is presented in Table 3 (additional marker data on the 90 cultivars not shown are available upon request from the senior author). Markers AP5659-1 and AP5930 both display unique marker alleles in germplasm carrying the *Pi-z* gene (see Table 3 for allele size results). For AP5659-3, the most closely linked marker to *Pi-z*, all germplasm with *Pi-z* carry the 223 nt allele, although several medium and long grain cultivars without *Pi-z* carry this same allele. Marker AP5659-5 shows a 277 nt allele for all germplasm with *Pi-z*, although this marker allele was also found in accession Pi-9, which carries the *Pi9(t)* gene, another resistance allele at the *Pi-z* locus (Liu et al. 2002). For marker RM527, the 215 nt allele is found in all *Pi-z* germplasm, but is also found in germplasm not carrying *Pi-z*, particularly in cultivars related to 'L-202'. Marker AP4791 shows good association with *Pi-z*, where nearly all germplasm carrying the *Pi-z* gene carry the 287 nt allele. However, 'Jefferson' (PI 593892) carries *Pi-z* and the 290 nt allele for AP4791, which is common in germplasm not carrying *Pi-z*. AP5413 shows a 338 nt allele for all *Pi-z* germplasm, but a small number of antiquated long grain accessions found in USA germplasm (Edith, Honduras, and Lady Wright; data from the 90 accessions not shown) that do not have *Pi-z* also carry this allele.

Two distinctive SSR marker haplotypes, therefore, were seen in germplasm carrying the *Pi-z* gene (Table 3), with a total of 42 SSR haplotypes detected in the 130 rice accessions studied (data not shown). One *Pi-z* haplotype, found in Zenith, is most common in germplasm carrying the *Pi-z* gene. The second *Pi-z* haplotype, which only differs from the Zenith haplotype at marker AP4791, is found in Jefferson and its derivatives, 'Cala' (PI 633972) and 'Presidio' (PI 636465), which carry *Pi-z*. The first cultivars developed in the USA having *Pi-z* resistance and markers are Zenith and Magnolia, although Magnolia appears to be heterogeneous for the *Pi-z* gene.

Both of the *Pi-z* haplotypes are unique resistance haplotypes, having different marker alleles than cultivars carrying the *Pi-z*^{t}, *Pi2(t)*, or *Pi9(t)* genes. To address the allele-specificity of these markers, it can be noted that only one marker shares the same SSR allele between *Pi2(t)* and *Pi-z* (AP5669-3) or between *Pi9(t)* and *Pi-z*

Table 3. SSR marker alleles and presence of the *Pi-z* gene in USA and international rice germplasm.

Variety	RM527	AP4791	AP5930	AP5659-5	AP5659-3	*Pi-z*[a]	AP5659-1	AP5413
Basmati 370	235[b]	300	153	388	227	–	202	329
Bengal	215	287	159	277	223	+	220	338
Cala	215	290	159	277	223	+	220	338
Cocodrie	215	303	147	296	298	–	202	329
Cypress	215	303	147	296	298	–	202	329
Dixiebelle	235	288	153	288	227	–	202	329
Drew	235	288	153	288	227	–	202	356
IR-64	228	290	147	292	223	–	206	329
Jefferson	215	290	159	277	223	+	220	338
Katy	231	288	153	288	227	–	202	356
KDM-105	219	292	147	292	227	–	204	303
L-202	215	303	147	296	298	–	202	329
Lafitte	215	287	159	277	223	+	220	338
Lemont	235	288	153	288	227	–	202	329
M-202	231	296	147	279	223	–	204	325
M-204	231	296	147	279	223	–	204	325
Magnolia	215	303	147	296	223/227[c]	±	220/204[c]	334
Mars	215	287	159	277	223	+	220	338
Maybelle	235	288	153	288	227	–	202	329
Mercury	215	287	159	277	223	+	220	338
Nipponbare	231	312	153	288	227	–	202	334
Panda	215	287	159	277	223	+	220	338
Pi-2	221	292	147	292	223	*Pi2(t)*	202	293
Pi-9	219	290	168	277	298	*Pi9(t)*	202	na
Presidio	215	287	159	277	223	+	220	338
Saber	235	288	153	288	227	–	202	356
Saturn	215	287	159	277	223	+	220	338
Te-Qing	219	290	147	292	223	–	202	293
Toride 1	219	288	173	309	227	*Pi-z*^{t}	206	311
Vista	215	287	159	277	223	+	220	338
Wells	231	288	153	288	227	–	202	356
Zenith	215	287	159	277	223	+	220	338

Markers are shown from left to right in the order they are found on the physical map of rice chromosome 6.

[a]'+' indicates presence of the *Pi-z* gene, '–' indicates no *Pi-z* gene, and '*Pi_*' indicates presence of other resistance alleles at the *Pi-z* locus. The *Pi-z* gene is shown between markers AP5659-1 and AP5659-3, although it could be found in between markers AP5659-3 and AP5659-5 (see Figure 1).

[b]Marker alleles displayed as nt chain length obtained from ABI3100 capillary electrophoresis instrument output. A null allele is shown as 'na'.

[c]Two alleles present in cultivar.

(AP5659-5), and no SSR alleles are shared in common between *Pi-z* and *Pi-z*^{t} (Table 3). Since the resistance spectra of *Pi-z*, *Pi-z*^{t}, *Pi2(t)*, and *Pi9(t)* are different for USA *Magnaporthe grisea* isolates (Table 4), it is not surprising that different SSR haplotypes are found in the *Pi-z* region.

Discussion

Repeated scanning of DNA sequence information for SSR sequences in the region surrounding the *Pi-z* locus was successfully employed to identify candidate SSR markers linked to the *Pi-z* gene. Nevertheless, an empirical process of trial and error screening for successful marker amplification and parental polymorphism testing was needed to identify the few primers (14 of 72 tested, or 19.4%) that were useful for more than one of the genetic crosses analyzed.

SSR markers tightly linked to the *Pi-z* gene, AP5659-1, AP5659-3, and AP5659-5, have been identified using 977 progeny lines segregating for the *Pi-z* gene from three separate crosses. These markers map to the same region as the *Pi-z* SNP markers identified by Hayashi et al. (2004), and

Table 4. Disease resistance reactions of resistance genes at the *Pi-z* locus inoculated with eight USA *Magnaporthe grisea* isolates.

Pi gene	Blast race							
	IB-45	IB-49	IB-54	IC-17	IE-1	IE-1k	IG-1	IH-1
Pi-z	S[a]	S	S	R	R	R	R	R
Pi-z^t	S	S	S	S	nd	nd	R	R
Pi2(t)	R	S	R	S	S	S	M	R
Pi9(t)	R	R	R	R	R	R	R	R

[a]Disease reactions having a rating of 0–2 are listed as 'R', 3–4 as 'M', 5 or more as 'S', and no data taken as 'nd'.

offer an alternative (non-SNP) type of marker for the *Pi-z* gene. Since these SSR markers are also closer to the *Pi-z* gene than the *Pi-z* SNP markers shown in Hayashi et al. (2004) and the *Pi-z* SSR markers identified by Conaway-Bormans et al. (2003), researchers can expect less recombination to occur between these markers and the *Pi-z* gene. The small differences in size between most of the resistant and susceptible alleles require the use of polyacrylamide gel or capillary electrophoresis systems to score these SSR markers, although marker AP5659-1 shows large enough size differences (14–18 nt) to be scored in high percentage agarose gel analyses.

Our mapping of the *Pi-z* gene to a 57 kb region on PAC AP005659 appears to be in complete agreement with the physical mapping of the *Pi2(t)* resistance factor at this same locus by Zhou et al. (2004). Our research does not indicate how many RGAs exist in the *Pi-z* gene cluster for the region flanked by these markers, since the genome sequence of Nipponbare may not reflect the structure of the *Pi-z* gene cluster itself. The number and arrangement of RGA elements for any one allele at this locus can vary considerably (Zhou et al. 2004), but the marker placement suggests that the genetic factors conferring the resistance reactions of the *Pi-z* and the *Pi2(t)* alleles should be in close physical distance. Preliminary analysis of a cross between Zenith and Pi-2, which carry the *Pi-z* and *Pi2(t)* resistance genes, respectively, indicate that the genetic factors encoding their separate resistance reactions are not the same, but are very tightly linked (results not shown).

The *Pi-z* markers reported here provide rice breeders and geneticists a valuable tool for marker aided selection of the *Pi-z* gene. Most of the *Pi-z* marker alleles are unique for the *Pi-z* gene, making several markers available for selection of the *Pi-z* gene within most genetic backgrounds. Alternatively, these *Pi-z* markers could be used for the selection of the *Pi2(t)*, *Pi9(t)*, or *Pi-z*^{t} resistance genes, since these are alleles found at the same locus. The utility of these markers, along with the markers of Conaway-Bormans et al. (2003), has already been demonstrated in selection of the *Pi-z* gene during the development of Cala and Presidio, two long grain cultivars released for production in the southern USA (McClung, personal communication), and of 'M-207', the first medium grain cultivar developed for California production having blast resistance (Johnson, personal communication).

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